Journal of Antimicrobial Chemotherapy Advance Access published May 3, 2014

Journal of Antimicrobial Chemotherapy

J Antimicrob Chemother
doi:10.1093/jac/dku139

Emergence of genetically unrelated NDM-1-producing *Acinetobacter pittii* strains in Paraguay

Fernando Pasteran[1], Mario Martinez Mora[2], Ezequiel Albornoz[1], Diego Faccone[1], Rossana Franco[2], Juana Ortellado[3], Nancy Melgarejo[2], Sonia Gomez[1], Irma Riquelme[3], Jorge Matheu[4], Pilar Ramon-Pardo[4] and Alejandra Corso[1]*

[1]*Servicio Antimicrobianos, Instituto Nacional de Enfermedades Infecciosas (INEI), ANLIS 'Dr Carlos G. Malbrán', Ciudad Autónoma de Buenos Aires, Argentina;* [2]*Servicio Antimicrobianos, Laboratorio Central de Salud Pública, Asunción, Paraguay;* [3]*Centro Materno Infantil-Hospital de Clínicas, Universidad Nacional de Asunción, San Lorenzo, Departamento Central, Paraguay;* [4]*Antimicrobial Resistance and Infection Control Program, International Regulations, Alert and Response and Epidemic Diseases and Water Borne Diseases, Communicable Diseases and Health Analysis (CHA), Pan American Health Organization/World Health Organization, Washington, DC, USA*

*Corresponding author. Tel/Fax: +54-11-4303-2812;
E-mail: acorso@anlis.gov.ar

Keywords: carbapenemases, resistance, paediatric

Sir,

The New Delhi metallo-β-lactamase (NDM-1) was initially identified in *Escherichia coli* and *Klebsiella pneumoniae* isolates in Sweden, from a patient previously hospitalized in India.[1] To date, NDM producers in Latin America have been scarce, and associated with species of Enterobacteriaceae from Guatemala, Mexico, Colombia and Brazil, although in Honduras it was reported in *Acinetobacter baumannii*.[2–6] Here, we report two genetically unrelated NDM-1-producing *Acinetobacter pittii* isolates identified in Paraguay.

Since 1996, the Pan American Health Organization (PAHO) has supported a regional surveillance system, the Antimicrobial Resistance Surveillance Network in Latin America (ReLAVRA), that includes 794 laboratories from 20 Latin American countries, including their respective reference laboratories.[7] This network provides reliable, timely and reproducible microbiological data in order to improve patient care. A regional protocol for the detection of carbapenemases has been harmonized and implemented through ReLAVRA. Briefly, metallo-β-lactamase (MBL) production is suspected in isolates that exhibit decreased susceptibility to carbapenems (CLSI criteria) and a positive synergy test result between a disc containing 10 μg of imipenem and a disc containing 750 μg of EDTA plus 1900 μg of sodium thioglycolate.[8]

During 2012, following the ReLAVRA algorithm, the National Health Laboratory of Paraguay confirmed an MBL phenotype in two *Acinetobacter* spp. isolates recovered from a single hospital. This phenotype had not previously been observed in *Acinetobacter* spp. from Paraguay. The first case was a 7-year-old patient admitted in July because of acute encephalitis. After 2 months of hospitalization, an *Enterobacter cloacae* extended-spectrum β-lactamase producer and *Acinetobacter* M15274 were recovered from the CSF obtained through a ventricular shunt. The patient received multiple treatment regimens, including trimethoprim/sulfamethoxazole, ciprofloxacin and amikacin, which resulted in clinical and microbiological cure. The patient died after 4 months of hospitalization due to non-infectious causes. The second case was a 2-year-old patient with a diagnosis of acute lymphocytic leukaemia who was admitted in November. Two weeks later the patient developed sepsis, and *Acinetobacter* M15373 was isolated from a blood culture. The patient showed clinical improvement after treatment with meropenem plus amikacin and was discharged alive after 50 days. Both patients were hospitalized in the same oncology ward but 4 months apart from each other. Remarkably, the patients had no history of travelling.

Strains were submitted to the Regional Reference Laboratory (Servicio Antimicrobianos, INEI, ANLIS 'Dr Carlos G. Malbrán') for further characterization. Strains were identified as *A. pittii* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF; Bruker, Germany); the 10 most probable database matches were all consistent with *A. pittii*. Antimicrobial susceptibility testing revealed an identical resistance profile in both *A. pittii* isolates, except for ampicillin/sulbactam and quinolones (Table 1). EDTA reduced the carbapenem MICs by at least three dilutions in both strains, suggesting the presence of MBLs (Table 1). The modified Hodge test gave negative results with meropenem but was positive (weakly) with imipenem.

In both isolates, PCR screening followed by DNA sequencing detected the presence of $bla_{\text{NDM-1}}$. PCRs targeting other β-lactamase genes (bla_{VIM}, bla_{IMP}, bla_{SPM}, bla_{KPC}, $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24/40}}$, $bla_{\text{OXA-48}}$, $bla_{\text{OXA-51}}$, $bla_{\text{OXA-58}}$, $bla_{\text{OXA-143}}$, $bla_{\text{CTX-M}}$, bla_{SHV} and bla_{TEM}) were negative. Sequencing of a 2.5 kb fragment surrounding $bla_{\text{NDM-1}}$ from both strains revealed 100% identity to the sequence reported for *Acinetobacter lwoffii*, where IS*Aba125* was located upstream of $bla_{\text{NDM-1}}$, followed by Δ*trpF* and *tat*.[9,10] The genes that follow to the 3′ end, Δ*groES*, *groEL*, IS*CR27* and the second IS*Aba25* were PCR mapped, revealing a Tn*125* composite transposon as previously reported in *A. pittii*.[11]

ApaI PFGE studies revealed that the *A. pittii* isolates were not clonally related (>10 bands of difference in the macrorestriction pattern). Multilocus sequence typing (MLST) was performed according to the MLST Database (http://www.pasteur.fr/recherche/genopole/PF8/mlst/). M15274 and M15373 displayed unique and novel sequence types (STs), designated ST320 and ST321, respectively. M15274 and M15373 shared only the *rplB* allele, confirming that they were genetically unrelated. Furthermore, these STs branched with other reported isolates belonging to the *A. pittii* genomic species, confirming the MALDI-TOF results.

Table 1. Antimicrobial susceptibility (MICs in mg/L)[a] of NDM-producing *A. pittii* clinical isolates and *A. baumannii* and *E. coli* transconjugant and recipient strains

	Clinical isolates		Transconjugant strains[b]		Recipient strains	
	A. pittii M15274	*A. pittii* M15373	*A. baumannii* M17176	*E. coli* M15694	*A. baumannii* ATCC 19606	*E. coli* J53
Imipenem[c]	>256	>256	>256	1	0.25	0.06
Imipenem/EDTA[d]	0.12	0.12	0.25	0.12	0.25	0.06
Meropenem[c]	>256	>256	>256	0.5	1	0.015
Meropenem/EDTA[d]	0.12	0.25	0.5	0.015	1	0.015
Ertapenem[c]	>256	>256	>256	2	1	0.25
Ampicillin/sulbactam	>16	4	>16	>16	≤1	≤1
Piperacillin/tazobactam	>64	>64	>64	>64	8	≤4
Aztreonam[c]	32	32	32	≤0.03	32	≤0.03
Cefotaxime[c]	>256	>256	>256	32	16	≤0.015
Ceftazidime[c]	>256	>256	>256	>256	8	≤0.06
Cefepime[c]	>256	>256	>256	16	16	0.015
Cefoxitin	>32	>32	>32	>32	≤8	≤8
Gentamicin	≤1	≤1	8	≤1	8	≤1
Amikacin	≤2	≤2	≤2	≤2	≤2	≤2
Nalidixic acid	16	≤2	4	≤2	4	≤2
Ciprofloxacin	1	≤0.25	1	≤0.25	1	≤0.25
Trimethoprim/sulfamethoxazole	≤2	≤2	>256	≤2	>256	≤2
Nitrofurantoin	>256	>256	>256	≤16	>256	≤16
Colistin	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
Tigecycline[c]	0.06	0.06	0.25	0.25	0.015	0.015

[a]Antimicrobial susceptibility testing according to CLSI standards.
[b]Transconjugant strains of *A. pittii* M15373.
[c]MICs were determined using agar dilution; MICs of other antibiotics were determined using the Vitek 2C (AST-N082 card).
[d]EDTA at a fixed concentration of 0.4 mM. The bla_{VIM-11}-producing *Pseudomonas aeruginosa* M5109 was used for quality control purposes.

The bla_{NDM-1} gene was transferred by biparental conjugation to either sodium azide-resistant *E. coli* J53 or *A. baumannii* ATCC 19606 from M15373 but not from M15274, conferring non-susceptibility to carbapenems on the recipient strain (Table 1). S1 nuclease digestion showed that both *A. pittii* and the transconjugant strains harboured a single plasmid of ~54 kb that hybridized with the bla_{NDM} probe. Plasmids gave negative results for all the Inc groups when assessed by PCR replicon typing.[12]

Additionally, further characterization of 23 contemporary carbapenem-resistant *Acinetobacter* spp. isolated in the same institution from November 2012 to March 2013 (clinical strains and patient swab samples) revealed a lack of MBL production and matched *A. baumannii* by MALDI-TOF.

A. pittii has recently been recognized as a key organism for the dissemination of NDM, since it has been associated with the dispersal of this carbapenemase in such diverse scenarios as food of animal origin and sewage, and has been responsible for both sporadic human infection and large outbreaks in hospital units.[10,13,14] Until now, non-*baumannii Acinetobacter* spp. expressing NDM have not been described in the American continent. These *A. pittii* clinical isolates are the first characterized NDM-1 producers from Paraguay. The origin of NDM-1-positive *A. pittii* in Paraguay remains unclear, since no history of travel to the suggested reservoirs of NDM was established for either patient. Our finding of bla_{NDM-1} on plasmids of identical 54 kb size in *A. pittii* strains with a heterogeneous clonal background suggests that bla_{NDM-1} most likely spread by the transfer of plasmids in *A. pittii*. Recent published data suggest that *A. pittii* could act as a potential NDM reservoir for Enterobacteriaceae based on the ease of plasmid transfer to *E. coli*,[11] as observed in one of the isolates reported here. Despite this ability, no further cases of NDM producers have so far been observed in the hospital.

In conclusion, the emergence of NDM in *A. pittii* constitutes a public health concern in Latin America, highlighting the relevance of an integrated surveillance of carbapenemase producers.

Acknowledgements

We thank platform Genotyping of Pathogens and Public Health (Institut Pasteur, Paris, France) for coding MLST alleles and profiles available at www.pasteur.fr/mlst, and Maria Soledad Ramirez PhD from the Instituto de Investigaciones en Microbiología y Parasitología Médica, Facultad de Medicina, Universidad de Buenos Aires and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina) for providing primers and reference strains.

Funding

This work was supported by the regular federal budget of the Ministry of Health of Argentina.

Transparency declarations

None to declare.

References

1 Yong D, Toleman MA, Giske CG *et al*. Characterization of a new metallo-β-lactamase gene, bla_{NDM-1}, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob Agents Chemother* 2009; **53**: 5046–54.

2 Pasteran F, Albornoz E, Faccone D *et al*. Emergence of NDM-1-producing *Klebsiella pneumoniae* in Guatemala. *J Antimicrob Chemother* 2012; **67**: 1795–7.

3 Barrios H, Garza-Ramos U, Reyna-Flores F *et al*. Isolation of carbapenem-resistant NDM-1-positive *Providencia rettgeri* in Mexico. *J Antimicrob Chemother* 2013; **68**: 1934–6.

4 Escobar Pérez JA, Olarte Escobar NM, Castro-Cardozo B *et al*. Outbreak of NDM-1-producing *Klebsiella pneumoniae* in a neonatal unit in Colombia. *Antimicrob Agents Chemother* 2013; **57**: 1957–60.

5 Waterman PE, McGann P, Snesrud E *et al*. Bacterial peritonitis due to *Acinetobacter baumannii* sequence type 25 with plasmid-borne New Delhi metallo-β-lactamase in Honduras. *Antimicrob Agents Chemother* 2013; **57**: 4584–6.

6 D'Alincourt Carvalho-Assef A, Pereira1 P, Mattos Albano R *et al*. Isolation of NDM-producing *Providencia rettgeri* in Brazil. *J Antimicrob Chemother* 2013; **68**: 2956–7.

7 Schmunis G, Salvatierra-Gonzalez S. Birth of a public surveillance system: PAHO combats the spread of antimicrobial resistance in Latin America. *The APUA Newsletter* 2006; **24**: 6–8.

8 Lee K, Lim Y, Yong D *et al*. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-β-lactamase-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J Clin Microbiol* 2003; **41**: 4623–9.

9 Hu H, Hu Y, Pan Y *et al*. Novel plasmid and its variant harboring both a bla_{NDM-1} gene and type IV secretion system in clinical isolates of *Acinetobacter lwoffii*. *Antimicrob Agents Chemother* 2012; **56**: 1698–702.

10 Fu Y, Du X, Ji J *et al*. Epidemiological characteristics and genetic structure of bla_{NDM-1} in non-*baumannii Acinetobacter* spp. in China. *J Antimicrob Chemother* 2012; **67**: 2114–22.

11 Bogaerts P, Huang T, Rezende de Castro R *et al*. Could *Acinetobacter pittii* act as an NDM-1 reservoir for Enterobacteriaceae? *J Antimicrob Chemother* 2013; **68**: 2414–5.

12 Carattoli A, Bertini A, Villa L *et al*. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005; **63**: 219–28.

13 Yang J, Chen Y, Jia X *et al*. Dissemination and characterization of NDM-1-producing *Acinetobacter pittii* in an intensive care unit in China. *Clin Microbiol Infect* 2012; **18**: E506–13.

14 Zhang R, Hu YY, Yang XF *et al*. Emergence of NDM-producing non-*baumannii Acinetobacter* spp. isolated from China. *Eur J Clin Microbiol Infect Dis* 2014; **33**: 853–60.